ACTA ABP BIOCHIMICA POLONICA

Epub: No 6641
Paper in Press
https://doi.org/10.18388/abp.2020_6641

Regular paper

Association of vitamin D with deoxyribonucleic acid (DNA) damage: A systematic review of animal and human studies

Mayang Indah Lestari[1,2], Krisna Murti[3]✉, Iche Andriyani Liberty[4], Zen Hafy[1], Violantina Linardi[5], Muhammad Khoirudin[5] and Tungki Pratama Umar[5]

[1]Doctoral Study Program in Biomedical Science, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia; [2]Department of Anesthesiology and Intensive Therapy, Faculty of Medicine, Sriwijaya University-Dr. Mohammad Hoesin General Hospital – Siti Fatimah General Hospital, Palembang, Indonesia; [3]Department of Anatomical Pathology, Faculty of Medicine, Sriwijaya University-Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia; [4]Department of Public Health, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia; [5]Research Assistant, Department of Anesthesiology and Intensive Therapy, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

Vitamin D has anti-proliferative, anti-inflammatory, and apoptotic abilities. Vitamin D deficiency can induce deoxyribonucleic acid (DNA) damage. The aim of the study was to create a systematic review to analyze the relationship between vitamin D and DNA damage in various populations. PubMed, Scopus, EbscoHost, Google Scholar, and Epistemonikos were used to identify literature regarding the relationship between vitamin D and DNA damage. Assessment of study quality was carried out by three independent reviewers individually. A total of 25 studies were assessed as eligible and included in our study. Twelve studies were conducted in humans consisting of two studies with experimental design and ten studies with observational pattern. Meanwhile, thirteen studies were conducted in animals (*in vivo*). It is found that the majority of studies demonstrated that vitamin D prevents DNA damage and minimizes the impact of DNA damage that has occurred ($p<0.05$). However, two studies (8%) did not find such an association and one research only found a specific association in the cord blood, not in maternal blood. Vitamin D has a protective effect against DNA damage. A diet rich in vitamin D and vitamin D supplementation is recommended to prevent DNA damage.

Keywords: vitamin D, DNA damage, observational studies, *in vivo* studies, systematic review

Received: 31 January, 2023; revised: 15 May, 2023; accepted: 16 May, 2023; available on-line: 17 June, 2023

✉e-mail: krisna.arinafril@unsri.ac.id

Abbreviations: γH2AX, phosphorylated histone H2AX; 1,25(OH)2D, 1,25-dihydroxyvitamin D; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; 25(OH)D, 25 hydroxy vitamin D; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; ROS, Reactive oxygen species; T2DM, Type 2 diabetes mellitus; UV, Ultraviolet; VDR, Vitamin D receptor

INTRODUCTION

Vitamin D and its receptors play an essential role in cancer development due to its anti-proliferative, anti-inflammatory, and apoptotic properties (Nair-Shalliker *et al.*, 2012a; Deuster *et al.*, 2017; Elhusseini *et al.*, 2018). Endogenous synthesis of vitamin D begins with cholesterol oxidation, producing pro-vitamin D3. In the skin, Ultraviolet B (UVB) from sunlight converts pro-vitamin D3 to pre-vitamin D3. Then, isomerization of pre-vitamin D3 is done, with vitamin D3 (cholecalciferol) as its main end product (Osmancevic *et al.*, 2015). Two hydroxylations by the enzymes vitamin D 25-hydroxylase (CYP27A1) and renal mitochondrial 1-hydroxylase (CYP27B1) are required to convert vitamin D3 to active 1,25(OH)2D3 (calcitriol). Calcitriol binds to vitamin D receptors (VDRs) belonging to the nuclear receptor family and forms a complex with RXR to regulate gene expression (Deuster *et al.*, 2017). VDRs are nuclear receptor superfamily members expressed in tumours to regulate cell cycle-related proliferation and angiogenesis (Khrisnan *et al.*, 2012; Christakos *et al.*, 2015). In addition, vitamin D has been shown in several studies to be effective in stimulating Deoxyribonucleic acid (DNA) synthesis in mature alveolar cells, modulating epithelial cell proliferation, and repairing injury (Usman *et al.*, 2021).

Furthermore, adequate vitamin D levels help to maintain DNA integrity. Vitamin D's role can be divided into two categories: primary functions that prevent DNA damage and secondary processes that regulate cell growth rate (Nair-Shalliker *et al.*, 2012a; Wenclewska *et al.*, 2019). Vitamin D is really crucial since its deficiency is associated with an increased frequency of chromosomal aberrations, sister chromatid exchanges, micronuclei formations, and alteration of comet assays (related to oxidative stress, hypoxic, and apoptotic process), some important indicators of DNA damage (Peng *et al.*, 2010; Nair-Shalliker *et al.*, 2012a; O'Callaghan-Gordo *et al.*, 2017; Liu *et al.*, 2019). The potential of vitamin D in reducing oxidative DNA damage in humans refers to clinical trials in which vitamin D supplementation reduced levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG), an oxidative damage biomarker found in colorectal epithelial crypt cells (Nair-Shalliker *et al.*, 2012a). Vitamin D administration has also been shown to reduce oxidative stress-induced damage and chromosomal aberration, prevent telomere shortening, and inhibit telomerase activity in animal models and cell lines (Siebert *et al.*, 2018). Vitamin D's secondary functions in preventing DNA damage include regulating poly-adenosine diphosphate/ADP-ribose polymerase activity on the DNA damage response pathway during the DNA lesion detection process. Vitamin D can also inhibit the replication of damaged DNA and regulate apoptosis, which promotes cell death (Nair-Shalliker *et al.*, 2012a).

Although vitamin D has long been discussed as one of the essential DNA protectors, a systematic review of the relationship between vitamin D and DNA damage has yet to be found. As a result, the purpose of this study is to use a systematic review approach to examine the association between vitamin D and DNA damage in

different populations of human studies and animal models.

METHODS

The manuscript was arranged using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 guidelines (Page *et al.*, 2021). All members approved the review panel's study procedure before conducting the literature search. The protocol has been registered in the International prospective register of systematic reviews (PROSPERO) with the registration number: CRD42023393054.

Literature retrieval

A comprehensive literature search was conducted across five databases to identify manuscripts on the relationship between vitamin D levels and DNA damage in human and animal subjects. The articles were discovered using Scopus, PubMed, EBSCOHost, Google Scholar, and Epistemonikos. Hand-searching was also conducted based on the included study bibliography to identify relevant publications that were not indexed in the previously reported databases (Umar *et al.*, 2022). There will be no restrictions on geographical region or gender. However, we restricted our search to studies published between January 2012 and January 2023. The investigation was completed by January 19th, 2023. To ensure its validity, the study must be published in English. The search terms for this study are available as Supplementary Table 1 at https://ojs.ptbioch.edu.pl/index.php/abp/.

Study selection

We sought animal and human studies investigating the link between vitamin D levels and DNA damage. Studies must demonstrate the influence of a single vitamin D substance administration on DNA damage (rather than a formulation containing multiple compounds) to be considered. The study was deemed ineligible if its design consisted of a literature review (e.g., systematic review, narrative review, scoping review), opinion, book chapter, and editorial. Meanwhile, included studies must have access to the full text. As a result, we excluded conference abstracts, posters, and unretrieved complete records. Duplicates were removed from the literature retrieval. Three independent reviewers (VL, MK, and ZH) assessed titles and abstracts (primary screening) using a semi-automated process aided by Rayyan QCRI software (Ouzzani *et al.*, 2016; Umar & Siburian, 2022). Following the completion of the first screening stage, the full text was assessed by two reviewers (IAL and TPU) to determine its eligibility for inclusion in the review. Any disagreements were discussed and resolved by a senior author (MIL) at any stage of manuscript evaluation.

Data extraction

The following information was extracted from the data: authorship, country of study, research participant data in the form of age, sex, and comorbidities (human), as well as experimental research data on animal type and age (animal studies), DNA damage parameters (comet tail length, tail DNA, and tail moment; phosphorylated Histone H2AX (γH2AX), 8-hydroxy-2'-deoxyguanosine (8-OHdG), chromosomal aberration, DNA damage score, micronuclei formation, telomere length, urinary cyclobutane thymine (T–T) dimer, and DNA repair indicator), and main findings. The information was recorded on the extraction sheet using Microsoft Office Excel 2019. Because of the vast diversity among included studies, the findings were presented as a qualitative synthesis rather than a meta-analysis.

Risk of bias analysis

The risk of bias (RoB) analysis was conducted for the animal studies using The Systematic Review Center for Laboratory Animal Experimentation's risk of bias (SYRCLE's RoB) tool. The RoB tool from SYRCLE contains ten items related to selection bias, performance bias, detection bias, reporting bias, and other biases (Hooijmans *et al.*, 2014). Meanwhile, we used three different scales for human studies: the Newcastle-Ottawa Scale (for observational studies), the Risk Of Bias In Non-randomized Studies – of Interventions (ROBINS-I), and Version 2 of the Cochrane risk-of-bias tool for randomized trials (ROB-2). The Newcastle Ottawa Scale is divided

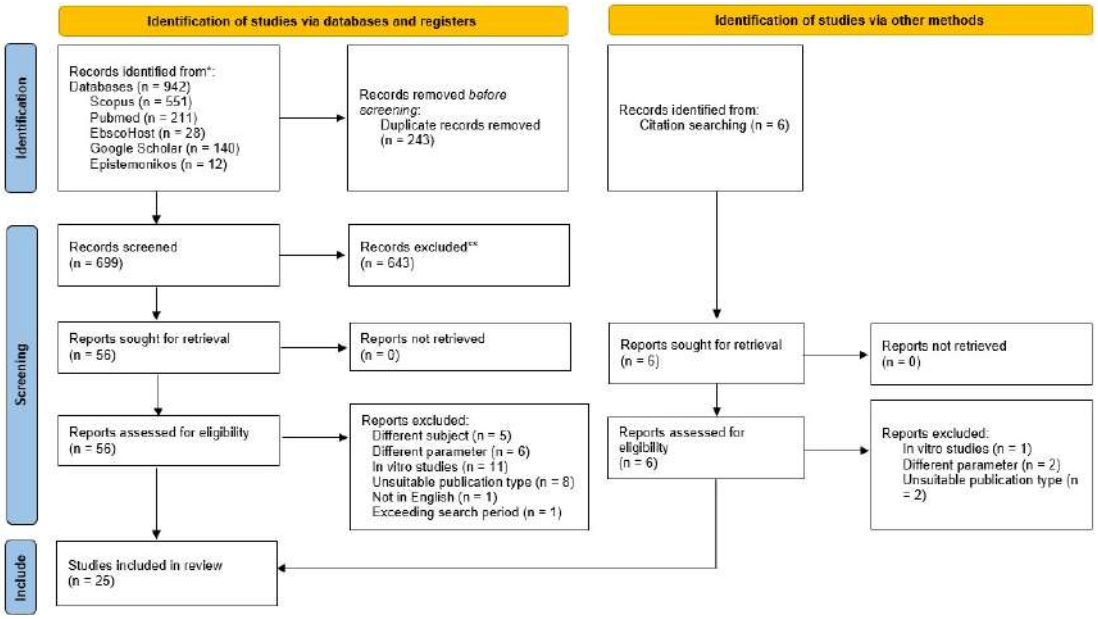

Figure 1. Study selection flow

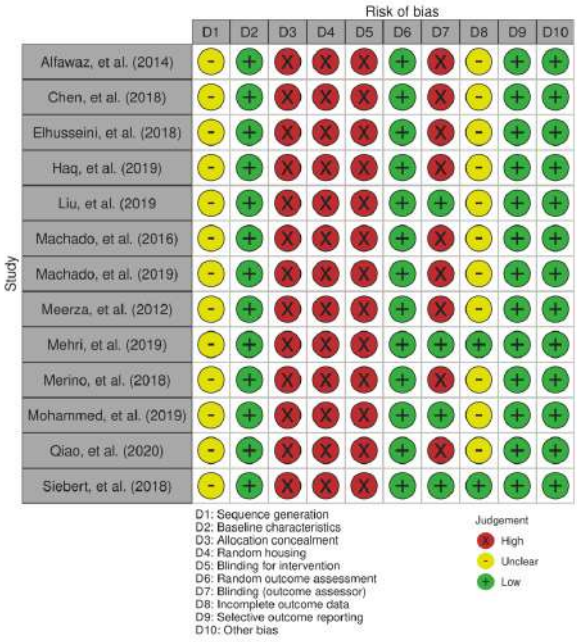

Figure 2. Risk of Bias for Animal Studies

into three major domains (selection, comparability, and outcome) (Liana *et al.*, 2022). In contrast, the ROBINS-I (Sterne *et al.*, 2016) and ROB-2 (Sterne *et al.*, 2019) are divided into several parts within the pre-intervention, intervention, and post-intervention stages. The evaluation was carried out independently by two authors (IAL and TPU). In the event of a disagreement, the decision is made by a senior author (KM).

RESULTS

The search strategy identified a total of 942 studies. The search query found 551 studies on SCOPUS, 140 on Google Scholar, 211 on PubMed, 28 on EbscoHost, and 12 on Epistemonikos. Following duplicate detection, 243 studies were excluded. Then, 699 studies entered title and abstract screening, where 56 studies were deemed eligible for full-text screening, which resulted in 24 studies being included in the final analysis. Meanwhile, six studies were identified from the citation search, and only one was finally contained. This process resulted in 25 selected studies (13 animal studies/*in vivo* and 12 human studies (two experimental studies and ten observational research)) considered in the final process of manuscript evaluation (Fig. 1).

Study characteristics

All of the included studies evaluated the association of vitamin D with DNA damage. Animal studies (Table 1) were done on the vitamin D-deficient diet (Chen *et al.*, 2018; Elhusseini *et al.*, 2018; Merino *et al.*, 2018), hypertension (Machado *et al.*, 2016, 2019), oxidative stress (Haq *et al.*, 2019; Liu *et al.*, 2019), and neurological disorder (Alfawaz *et al.*, 2014; Mehri *et al.*, 2020). Meanwhile, all of the following parameters were assessed only in one study: diabetes mellitus (Meerza *et al.*, 2012), high-fat diet (Merino *et al.*, 2018), kidney disease (Mohammed *et al.*, 2019), aging (Qiao *et al.*, 2020), and ovariectomy (Siebert *et al.*, 2018) model. There are four main DNA damage detection methods, including immunohistochemistry

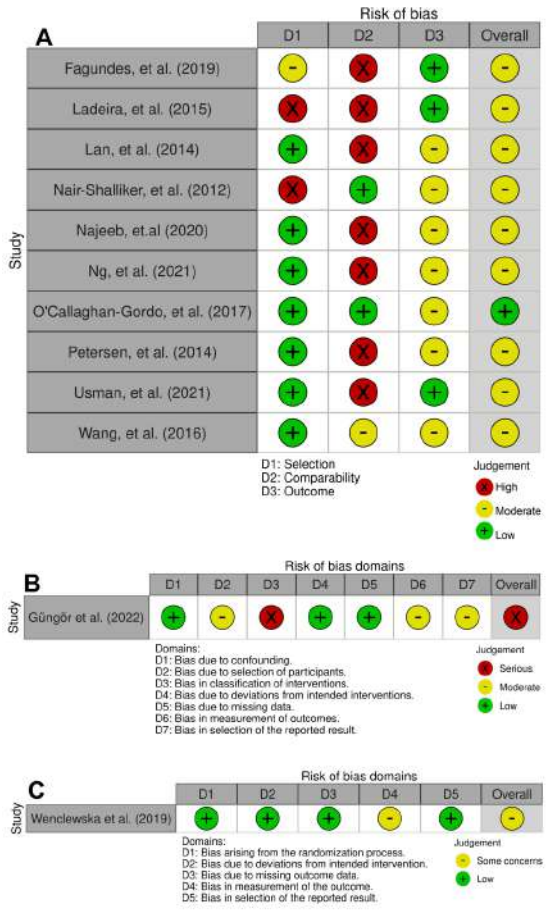

Figure 3. Risk of Bias for Human Studies.
A, Newcastle-Ottawa Scale; **B**, Risk of Bias in Non-randomised Studies – of Interventions (ROBINS-I); **C**, Version 2 of the Cochrane risk-of-bias tool for randomized trials (ROB-2)

(Chen *et al.*, 2018; Elhusseini *et al.*, 2018; Mohammed *et al.*, 2019), comet assay (alkaline comet assay) (Alfawaz *et al.*, 2014; Liu *et al.*, 2019; Machado *et al.*, 2016, 2019; Siebert *et al.*, 2018), Enzyme-linked immunosorbent assay (ELISA) (Haq *et al.*, 2019; Mehri *et al.*, 2020), and micronuclei detection (Liu *et al.*, 2019; Machado *et al.*, 2016). Meanwhile, other detection methods are flow cytometry (Merino *et al.*, 2018), gel electrophoresis (Haq *et al.*, 2019), Western blot (Qiao *et al.*, 2020) and reverse transcription polymerase chain reaction (RT-PCR) (Siebert *et al.*, 2018). Rodents, both mice and rats, were used as

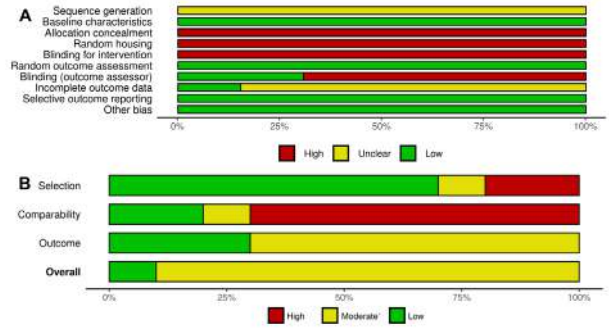

Figure 4. Risk of Bias Summary.
A, Animal studies; **B**, Human studies (limited to observational studies since experimental research only consisted of one study)

Table 1. Data extraction for animal studies

Author (year)	Country	Experimental animal	Disease model and inducer	Parameter	Method	Major findings
(Alfawaz *et al.*, 2014)	Saudi Arabia	Western Albino rats (±21 days, n=28)	Neurotoxicity by PPA	Tail length Tail DNA (%) Tail Moment (Unit)	Comet DNA assay	There is a potential impact of vitamin D in protecting and treating PPA neurotoxicity, while ameliorating the DNA-damaging effects of PPA. Prevention impact of vitamin D against PPA-induced DNA damage is more profound than its treatment effect
(Chen *et al.*, 2018)	China	1α(OH)ase$^{-/-}$and wild-type mice (120 pairs divided into four groups)	Vitamin D-deficient model	γH2AX 8-OHdG	Immunohistochemistry	There is an increase of DNA damage in 1,25(OH)2D3-deficient mice as measured by γH2AX and 8-OHdG on tumor cells according to immunohistochemistry
(Elhusseini *et al.*, 2018)	United States	Female C57BL/6 mice, (4–6 weeks old), control (n = 10), vitamin D-deficient diet group (n = 10)	Vitamin D-deficient model	γH2AX DNA repair (RAD50 and RAD51)	Immunohistochemistry	Vitamin D-deficient mice showed increased γ-H2AX in myometrial tissue compared to healthy controls (P<0.05) Vitamin D deficiency caused a significant reduction in the expression of DNA repair genes such as RAD50 (P<0.005) and RAD51 (P<0.005) compared to healthy controls.
(Haq *et al.*, 2019)	Saudi Arabia	Wistar Albino rats (1 week, n = 4)	Hydrogen peroxide (H_2O_2)	Chromosomal aberration (DNA fragmentation) 8-OHdG	Gel electrophoresis ELISA	Vitamin D, which was given for 24 hours prior to the induced oxidative stress by H_2O_2 significantly (p<0.001) reversed the deleterious and damaging effect of H_2O_2 alone as presented by DNA fragmentation percentage Significantly lower value of 8-OHdG following the administration of vitamin D+ H_2O_2 than H_2O_2 alone (p<0.05)
(Liu *et al.*, 2019)	China	Male mice (7–8 weeks)	CP	DNA damage score, total DNA in the tail (%), tail length, tail moment Micronuclei formation	Alkaline comet assay Buccal Micronuclei Cytome assay	Vitamin D3 suppressed CP-induced micronucleus formation in mice Buccal cells, with an alleviation range of 36.73–44.46% (p<0.05) Vitamin D3 injection for a dose of 5,000 IU significantly reduced CP-induced DNA damage, with a 46.6% decrease in tail DNA percentage (p<0.05), a 24.2% decrease in tail length (p<0.05), and 37.3% decrease in olive tail moment (P<.05). However, it is not significant at the 1,000 or 10,000 IU dose
(Machado *et al.*, 2016)	Brazil	Male Spontaneously hypertensive rats (SHR) and WKY (20 weeks) divided into six groups)	Hypertension model	DNA damage score, total DNA in the tail (%), tail length, tail moment Micronuclei formation	Alkaline comet assay Micronucleus test	Vitamin D3 deficient diet was able to increase the percentage of DNA damage in both SHR (49%) and WKY rats (54%) SHR rats with a vitamin D3 deficient diet showed a significant increase in the incidence of micronuclei formation in the bone marrow and peripheral blood (p<0.05)
(Machado *et al.*, 2019)	Brazil	Male Spontaneously hypertensive rats (SHR) and WKY (20 weeks) divided into six groups)	Hypertension model	Total DNA in the tail (%)	Alkaline comet assay	There was no significant difference in the percentage of DNA in comet tails (p>0.05) in the vitamin D deficiency group when compared to the control group. Vitamin D3 supplementation or deficiency did not significantly affect cardiac genotoxicity.
(Meerza *et al.*, 2012)	India	Female albino mice	Diabetes model by alloxan (200 mg/kgBW)	DNA tail length	Comet assay	Vitamin D-supplemented group showed a significant decrease in liver (21.80 ± 2.40 μm) and pancreatic (19.25 ± 1.90 μm) DNA tail length in diabetic mice
(Mehri *et al.*, 2020)	Iran	Wistar rats (200–240g, n=48)	Alzheimer's Disease by 5 μl of Aβ-containing solution	8-OHdG	ELISA	The level of DNA damage in Vitamin D and Aβ + Vitamin D groups in hippocampus and Vitamin D group of serum samples was significantly lower than that of the Aβ group (p < 0.0001)
(Merino *et al.*, 2018)	Chile	Male Sprague-Dawley rats (4 months, n=20 divided into four groups)	High-fat diet and vitamin-D deficient-diet	DNA Fragmentation	Flow cytometry	Vitamin D supplementation results in lower DNA fragmentation, either in the control or experimental group (p<0.05) The interaction between vitamin D deficiency and diet-induced obesity was significant in DNA fragmentation (p = 0.0359)

(Mohammed *et al.*, 2019)	Egypt	Male albino rats (8-10 weeks, n=24 divided into four groups)	Acute renal damage by gentamycin 100 mg/kgBW	8-OHdG	Immunohistochemistry	Vitamin D administration significantly reduces 8-OHdG immunohistochemical expression when compared to the control group ($p< 0.01$)
(Qiao *et al.*, 2020)	China	Cyp27b1$^{+/-}$ and wild-type mice (9 months)	Aging model	γ-H2AX	Western blot	1,25(OH)2D insufficiency increases γ-H2AX expression significantly ($p<0.001$) compared with the wild type mice
(Siebert *et al.*, 2018)	Brazil	Wistar Albino rats (90 days or 180 days, divided into four groups)	OVX	DNA damage index Telomere length	Alkaline comet assay RT-PCR	OVX significantly increases DNA damage ($p < 0.001$) when compared to control. Vitamin D alone decreased the DNA damage index ($p < 0.05$ and $p < 0.001$), but when associated with OVX (OVX + VIT D), partially reversed DNA damage induced by OVX ($p < 0.001$). Vitamin D did not change telomere length ($p > 0.05$), and when associated with OVX (OVX + VIT D), Vitamin D supplementation was able to reverse the observed telomere shortening ($p < 0.005$).

Abbreviation: γ-H2AX, γ phosphorylated form of the histone H2AX, 1,25(OH)2D3, 1,25-dihydroxyvitamin D3, 1α(OH)ase, 1alpha-Hydroxylase, 8-OHdG, 8-Hydroxyguanosine, Aβ, Amyloid beta, C57BL/6, C57 black 6, CP, Cyclophosphamide, Cyp27b1, Cytochrome p450 27B1, DNA, Deoxyribonucleic acid, ELISA, Enzyme-linked immunosorbent assay, H_2O_2, Hydrogen peroxide, IU, International unit, mg/kgBW, milligrams/kilograms body weight, OVX, Ovariectomy, PPA, Propionic acid, RAD50, DNA repair protein RAD50, RAD51, DNA repair protein RAD51, RT-PCR, Reverse transcription polymerase chain reaction, WKY, Wistar Kyoto rat

animal models in all studies. It was found that all DNA damage parameters are associated with vitamin D levels, except in one study which did not find significant difference in the percentage of DNA in comet tails in the vitamin D deficiency group when compared to the control group (Machado *et al.*, 2019). Another study also showed that the preventive impact of vitamin D supplementation is better than its treatment effect to ameliorate DNA damage (Alfawaz *et al.*, 2014).

Human research (Table 2) were conducted in experimental (randomized and non-randomized clinical trial) (Wenclewska *et al.*, 2019; Gungor *et al.*, 2022) and observational (cross-sectional and cohort) (Ladeira *et al.*, 2015; Lan *et al.*, 2014; Nair-Shalliker *et al.*, 2012b; Najeeb *et al.*, 2020; Ng *et al.*, 2021; O'Callaghan-Gordo *et al.*, 2017; Petersen *et al.*, 2014; Usman *et al.*, 2021; Wang *et al.*, 2016; Fagundes *et al.*, 2019) design. In human studies, the most commonly employed parameter is comet assay (comet tail length, DNA damage score, and percentage of DNA in comet tail) (Fagundes *et al.*, 2019; Lan *et al.*, 2014; Najeeb *et al.*, 2020; Ng *et al.*, 2021; Wang *et al.*, 2016; Wenclewska *et al.*, 2019) and micronuclei formation (buccal or lymphocyte) (Fagundes *et al.*, 2019; Ladeira *et al.*, 2015; Nair-Shalliker *et al.*, 2012b; O'Callaghan-Gordo *et al.*, 2017; Usman *et al.*, 2021). Other parameters are aniline blue staining (sperm DNA damage) (Gungor *et al.*, 2022), thymine dimer (Petersen *et al.*, 2014), and ELISA (urinary 8-OHdG) (Usman *et al.*, 2021). Several conditions were observed in the included studies, such as diabetes mellitus, cancer, obesity, vitamin D-deficient state, and infertility. A similar finding was also observed in animal studies when the majority of the investigations revealed a significant association between vitamin D and DNA damage status. However, a study on workers occupationally exposed to formaldehyde (Ladeira *et al.*, 2015) and on the general population (Nair-Shalliker *et al.*, 2012b) did not show a significant association between vitamin D level and micronuclei formation, while another study only found the association in cord blood but not in maternal blood (O'Callaghan-Gordo *et al.*, 2017). Another research also did not present any association between vitamin D level and comet assay result as the DNA damage marker in the general population (Wang *et al.*, 2016).

Risk of Bias

All of the animal studies included in the analysis followed a similar pattern (Figs 2 and 4), with a low risk of bias on baseline characteristics, random outcome assessment, selective outcome reporting, and other biases. However, it is noteworthy that allocation concealment, random housing, and intervention blinding were not met by all studies, resulting in a high risk of bias. Meanwhile, only four studies (Liu *et al.*, 2019; Mehri *et al.*, 2020; Mohammed *et al.*, 2019; Siebert *et al.*, 2018) have a low risk of bias for outcome assessor blinding, and only two studies (Mehri *et al.*, 2020; Siebert *et al.*, 2018) have a low risk of bias for incomplete outcome data analysis.

We assessed the risk of bias in human studies (Fig. 3) using three scales: NOS, ROBINS-I, and ROB-2. Most studies (10/12; 83.33%) have moderate/some concerns about bias. A non-randomized study, on the other hand, runs the risk of bias due to insufficient intervention classification. Meanwhile, there is only one study (O'Callaghan-Gordo *et al.*, 2017) that has a low overall risk of bias. According to the summary graph (Fig. 4) on observational studies, 70% have a high risk of bias on comparability due to a lack of explanation on confound-

Table 2. Data extraction for human studies

Author (year)	Location	Design	Age	% Male	Population	Method	Outcome
(Fagundes *et al.*, 2019)	Brazil	Prospective cohort	62.11 ± 9.64[a,d]	37	75 patients with type 2 diabetes mellitus who were given supplementation of vitamin D3 4000 IU/day for 8 weeks	Comet assay Buccal micronucleus cytome assay	Decreased DNA damage index (comet assay) ($p \leq 0.05$) and micronuclei formation ($p \leq 0.05$) following supplementation with vitamin D3 and a wash-out period There is a negative correlation between DNA damage index and vitamin D levels ($r=0.2569$; $p<0.0001$) but not in micronuclei.
(Gungor *et al.*, 2022)	Turkey	Non-RCT	34.67±4.01[a,c]	100	58 men with unexplained infertility (+50 controls)	Aniline blue staining (sperm DNA damage)	There was a negative and significant correlation between vitamin D levels and sperm DNA damage ($r = -0.605$, $p<0.001$)
(Ladeira *et al.*, 2015)	Portugal	Cross-sectional	39.64 ± 11.5[a,c]	65.45	55 workers occupationally exposed to Formaldehyde (+80 controls)	CBMN assay (buccal and lymphocyte)	Vitamin D has no association with the frequency of micronuclei (lymphocytes or buccal cells) in workers exposed to formaldehyde
(Lan *et al.*, 2014)	China	Cross-sectional	50 ± 10[a,c]	50	16 patients with severe asthma and vitamin D <30ng/ml (+16 controls and 16 patients with vitamin D >30ng/ml)	Comet assay (DNA damage score)	The total DNA damage score for a subject with Vitamin D deficiency was significantly increased compared to the scores in Vitamin D sufficiency ($p = 0.002$).
(Nair-Shalliker *et al.*, 2012b)	Australia	Cross-sectional	46.0 (27.1-61.4)[b,d]	NA	207 participants	CBMN assay	There is no association between log serum 25(OH)D concentration and log-transformed frequency of any CBMN-cyt assay biomarker ($p=0.3$)
(Najeeb *et al.*, 2020)	Iraq	Cross-sectional	61.87 ± 12.77[a,c]	48.89	45 cancer patients (+ 35 controls)	Comet assay	Correlation between Tail DNA% and plasma vitamin D is significant in cancer patients ($r^2=0.3707$; $p<0.0001$) and control ($r^2=0.2824$; $p<0.001$) with higher damage at lower vitamin D level
(Ng *et al.*, 2021)	Malaysia	Cross-sectional	29.96 ± 0.63[a,c]	0	134 participants (47 obese, 87 non-obese)	Alkaline comet assay	Multivariate analysis revealed that individuals with serum 25(OH)D level of ≥ 31 nmol/L had a significantly lower tail moment (1.06 ± 0.22 nmol/L vs. 2.37 ± 0.60 nmol/L; $p = 0.029$) and tail olive moment (2.36 ± 0.24 nmol/L versus 3.41 ± 0.46 nmol/L; $p = 0.031$) compared to those with lower serum 25(OH)D level, in the obese group
(O'Callaghan-Gordo *et al.*, 2017)	Spain	Cross-sectional	NA	NA	344 participants (173 mothers and 171 newborns)	CBMN assay	In cord blood, 25(OH)D insufficient values (<50 nmol/L) were associated with increased lymphocyte micronuclei frequency (adjusted IRR = 1.32 (1.00, 1.72)), but not in maternal blood
(Petersen *et al.*, 2014)	Denmark	Cross-sectional	39.74 ± 7.22[a,d]	46.48	71 participants	Urinary cyclobutane thymine (T–T) dimers	The association between cyclobutane thymine dimers (T–T dimers) and vitamin D is significant ($r^2 = 0.76$; $p<0.0001$), strongly indicating that the harmful DNA effects of ultraviolet radiation are unavoidable
(Usman *et al.*, 2021)	United Kingdom	Cross-sectional	14.60 ± 2.05[a,c]	45.28	132 adolescents (53 obese, control: 59 non-obese)	Buccal micronucleus cytome assay (buccal epithelial cells) ELISA (measures 8-OHdG from urine)	Vitamin D has significant correlation with 8-OHdG ($r^2=-0.245$; $p<0.01$) and buccal micronuclei ($r^2=-0.305$; $p<0.01$)
(Wang *et al.*, 2016)	China	Cross-sectional	20.69 ± 1.50[a,d]	36.36	121 participants (44 males, 77 females)	Comet Assay IV Lite scoring system	No significant correlation was observed between 25(OH) D level and DNA damage ($r = –0.0824$; $P > 0.05$).
(Wenclewska *et al.*, 2019)	Poland	RCT	63.43 ± 1.57[a,c]	29.17	92 people with vitamin D deficiency (intervention: 48 people, control: 44 people (14 with T2DM, 30 healthy)	Comet assay (peripheral lymphocyte)	The percentage of DNA in the tail decreased in the intervention group when compared to the control group, either with or without T2DM ($p<0.05$) DNA oxidative parameters (Fpg) decreased in the intervention group (113.63 ± 4.26 vs. 104.19 ± 3.06; $p<0.05$), especially in the T2DM group when compared to the control group ($p<0.01$).

Abbreviation: 25(OH)D, 25 hydroxy vitamin D, 8-OHdG, 8-Hydroxyguanosine, T–T dimers, cyclobutane thymine dimers, CBMN, cytokinesis-block micronuclei, Cyp27b1, Cytochrome p450 27B1, DNA, Deoxyribonucleic acid, ELISA, Enzyme-linked immunosorbent assay, H_2O_2 Hydrogen peroxide, IU, International unit, IRR, Incidence rate ratio, RCT, Randomized controlled trial, T2DM, Type 2 diabetes mellitus. Data was presented as: (a) mean ± SD or (B) median (min-max), (c) exposed mean, (d) overall mean

ing control. However, regarding selection, 70% of the studies have a low risk of bias, and 20% of the included research has a high risk of bias. Most studies have a moderate risk of bias in outcome assessment, primarily due to non-blinding outcome assessment.

DISCUSSION

DNA damage can be divided into two types, endogenous and exogenous. Endogenous DNA damage stems from chemically active DNA involved in hydrolytic and oxidative reactions with air and reactive oxygen species (ROS), which are naturally present in cells. On the contrary, exogenous DNA damage occurs due to the involvement of environmental, physical, and chemical substances such as UV and ionizing radiation, alkylating agents, and cross-linking agents (Chatterjee & Walker, 2018). Vitamin D is regarded as an essential factor in the status of DNA damage (Najeeb *et al.*, 2020). Vitamin D deficiency (plasma 25(OH)D <50 nmol/l) and severe deficiency (<30 nmol/l) have been associated with elevated oxidative stress, DNA damage promotion, and overall mortality (Wang *et al.*, 2016). The impact of vitamin D on DNA damage is prominent in several disorders, including hyperglycemia and cancer (Gabryanczyk *et al.*, 2021).

Hyperglycemia increases the production of free radicals and also induces DNA damage (Giacco & Brownlee, 2010). Studies conducted in patients with type 2 diabetes mellitus (T2DM) showed that vitamin D significantly prevented DNA damage and oxidative stress in patients with T2DM ($p<0.05$). A vitamin D-responsive element has been identified in the promotion region of the insulin receptor gene in human (Gikas *et al.*, 2009). Pancreatic cells express the nuclear receptor for 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), which modulates insulin action (Bland *et al.*, 2004). Furthermore, vitamin D minimizes insulin resistance by its effect on calcium and phosphorus metabolism along with the upregulation of the insulin receptor gene, as well as suppression of the synthesis of proinflammatory cytokines that contribute to insulin resistance, including interleukins and TNF-α due to its antioxidative properties (Wenclewska *et al.*, 2019; Maestro *et al.*, 2002; Talaei *et al.*, 2013).

In malignancy, vitamin D had positive functions as anti-proliferative, proapoptotic, anti-inflammatory, anti-angiogenesis, anti-metastatic, and anti-invasion as well as estrogen signaling inhibitor (Vuolo *et al.*, 2012; Deuster *et al.*, 2017; Wacker & Holiack, 2013). Calcitriol (the active form of vitamin D) inhibits the proliferation of many malignant cells by inducing cell cycle arrest and cell accumulation in the G0/G1 phase of the cell cycle. In cells, calcitriol causes G1/G0 arrest in a p53-dependent manner by increasing the expression of the cyclin-dependent kinase inhibitors p21Waf/Cip1 and p27Kip1, decreasing the activity of cyclin-dependent kinase 2 (CDK2), and causing hypo-phosphorylation. Calcitriol also increases the expression of p73, a homolog of p53, which is associated with the induction of apoptosis in several human and murine tumor systems. Suppression of p73 abrogates calcitriol-induced apoptosis and reduces the ability of calcitriol to enhance the cytotoxic effect of agents such as gemcitabine and cisplatin in a squamous cell carcinoma (SCC) model (Krishnan & Feldman, 2010; Khrisnan *et al.*, 2012). In the previous study, it was found that vitamin D deficiency is a risk factor for malignancy (cancer) and accelerates the invasion process (Najeeb *et al.*, 2020; Migliaccio *et al.*, 2022). The population of the 25 studies in this systematic review is diverse. Twelve studies conducted in humans analyzed DNA damage in patients with comorbid diseases such as T2DM, obesity, infertility, cancer patients, and the general population. Meanwhile, thirteen *in vivo* studies analyzed DNA damage using animal models with hypertension, ovariectomy, nephrotoxicity, vitamin D deficiency, and oxidative stress.

Several studies on vitamin D indicated that the vitamin has a beneficial impact on all organ systems of the human body. Both 25(OH)D and its hormonally active form, 1,25(OH)2D are vital for physiological functions, especially to reduce inflammation and excessive cellular oxidative stress. The 1,25(OH)2D hormone or calcitriol modulates cell proliferation through direct and indirect pathways, such as by the inhibition of the transcription factor, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which is associated with an elevation of oxidative stress and cellular response to inflammation and injury (Tilstra *et al.*, 2012). Due to suppression of NF-κB activation, calcitriol helps to reduce chronic inflammation (Myszka & Klinger, 2014). However, more research into the relationship between DNA damage, oxidative stress, and vitamin D is required.

Vitamin D receptor (VDR) is found in testicular tissue, prostate, and spermatozoa. In addition, the intense metabolism of vitamin D in the male reproductive system and increased expression of VDR in the neck of the sperm cause males to require vitamin D for functionally active sperm (Jensen *et al.*, 2011). Incubation of semen samples with vitamin D for 30 minutes led to a significant increase in sperm velocity parameters. This progressive increase in motility is due to vitamin D-dependent calcium release and subsequent cyclic AMP/protein kinase A (cAMP/PKA) activation and Adenosine triphosphate (ATP) production (Gunter *et al.*, 2004). There was a significant negative correlation ($p<0.05$) between vitamin D and sperm DNA damage in this systematic review. After binding to the VDR receptor, vitamin D initiates slow genomic effects by stimulating the release of ligand-activated transcription factors in the nucleus. In unexplained infertile patients with vitamin D deficiency, sperm DNA damage may occur due to delayed genomic effects (Jurutka *et al.*, 2001).

Studies in various animal models showed that vitamin D exerts a protective effect on DNA. Vitamin D can reduce the DNA damage index (percentage of DNA in comet tails assessed from comet tests). In addition, in genomic instability animal models induced by cyclophosphamide, vitamin D can reduce the frequency of micronuclei formation (a marker of DNA damage). Besides the percentage of DNA in comet tails and the frequency of micronuclei, DNA damage can also be assessed by the levels of 8-OHdG, a marker of oxidative DNA damage (Smith *et al.*, 2005). Elevated levels of 8-OHdG in rat animal models are also associated with a complete loss of VDR expression. Because the expression of VDR depends on the availability of 1,25(OH)2D, the loss of VDR suggests that there may be a role for 1,25(OH)2D in protecting cells against hyperproliferation and oxidative DNA damage (Kállay *et al.*, 2002; Nair-Shalliker *et al.*, 2012a). Decreased levels of 8-OHdG after vitamin D supplementation was proven in the animal model studies (Haq *et al.*, 2019; Mohammed *et al.*, 2019). The results of studies in animal models (*in vivo*) are in line with those of studies in cells (*in vitro*) (Chen *et al.*, 2018; Liu *et al.*, 2019). These outcomes can confirm that vitamin D has a protective effect on DNA.

Various parameters of oxidative stress are also presented in this systematic review. In human studies, vitamin D supplementation led to a significant decrease in NO and total thiols and an increase in the concentration of reduced glutathione (GSH) leading to a decrease in oxidative processes in cells (Fagundes *et al.*, 2019). In a study of animal models with hypertension, vitamin D was not significantly associated with DNA damage. However, vitamin D3 deficiency alters the level of Thiobarbituric Acid Reactive Substance (TBARS) in a mouse model of spontaneous hypertension, which is an indicator of Reactive Oxygen Species (ROS)-initiated peroxidation of unsaturated fatty acids in membrane lipids and alters the permeability, fluidity, and integrity of the plasma membrane (Potter *et al.*, 2011). Lipid peroxidation predisposes patients to conditions such as hypertension and thromboembolic (Yavuzer *et al.*, 2016). Vitamin D can reduce oxidative stress that occurs in cells thereby reducing DNA damage.

This systematic review proved that vitamin D protects against DNA damage. However, there are some limitations to this systematic review. First, the study populations are largely heterogeneous with different diseases and DNA damage parameters; thus, the results can be biased. In addition, human studies are still sparse (only one randomized controlled trial/RCT) and mainly with a moderate RoB. Consequently, the application of the results to humans still needs to be considered. Further studies with randomized controlled trial designs are expected in the future to increase the strength of evidence.

CONCLUSION

There is a significant association between vitamin D and DNA damage. However, although the majority of studies have found that vitamin D has a protective effect against DNA damage, other research found contradictory findings. Thus, the need of further investigations with stricter criteria must be commenced. Nevertheless, it is safe to conclude that a diet with sufficient vitamin D content and supplementation (more than 1000 IU/day, preferably about 2000–5000 IU/day) is recommended to prevent DNA damage and oxidative stress in cells.

REFERENCES

Alfawaz HA, Bhat RS, Al-Ayadhi L, El-Ansary AK (2014) Protective and restorative potency of vitamin D on persistent biochemical autistic features induced in propionic acid-intoxicated rat pups. *BMC Complement. Altern. Med.* **14**: 416. https://doi.org/10.1186/1472-6882-14-416

Bland R, Markovic D, Hills CE, Hughes S V., Chan SL, Squires PE, Hewison M (2004) Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J. Steroid Biochem.* 121–125. https://doi.org/10.1016/j.jsbmb.2004.03.115

Chatterjee N, Walker GC (2018) Mechanisms of DNA damage, repair, and mutagenesis. *Environ. Mol. Mutagen.* **58**: 235–263. https://doi.org/10.1002/em.22087.Mechanisms

Chen L, Yang R, Qiao W, Yuan X, Wang S, Goltzman D, Miao D (2018) 1,25-Dihydroxy vitamin D prevents tumorigenesis by inhibiting oxidative stress and inducing tumor cellular senescence in mice. *Int. J. Cancer* **143**: 368–382. https://doi.org/10.1002/ijc.31317

Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G (2015) Vitamin D: Metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol. Rev.* **96**: 365–408. https://doi.org/10.1152/physrev.00014.2015

Deuster E, Jeschke U, Ye Y, Mahner S, Czogalla B (2017) Vitamin D and VDR in gynecological cancers-A systematic review. *Int. J. Mol. Sci.* **18**: 2328. https://doi.org/10.3390/ijms18112328

Elhusseini H, Elkafas H, Abdelaziz M, Halder S, Atabiekov I, Eziba N, Ismail N, El Andaloussi A, Al-Hendy A (2018) Diet-induced vitamin D deficiency triggers inflammation and DNA damage profile in murine myometrium. *Int. J. Womens. Health* **10**: 503–514. https://doi.org/10.2147/IJWH.S163961

Fagundes G, Macan T, Rohr P, Damiani A, Rocha F Da, Pereira M, Longaretti L, Vilela T, Ceretta L, Mendes C, Silveira P, Teixeira J, Andrade V de (2019) Vitamin D3 as adjuvant in the treatment of type 2 diabetes mellitus: modulation of genomic and biochemical instability. *Mutagenesis* **34**: 135–145. https://doi.org/10.1093/mutage/gez001

Gabryanczyk A, Klimczak S, Szymczak-Pajor I, Śliwińska A (2021) Is vitamin D deficiency related to increased cancer risk in patients with type 2 diabetes mellitus? *Int. J. Mol. Sci.* **22**: 6444. https://doi.org/10.3390/ijms22126444

Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. *Circ. Res.* 1058–1070. https://doi.org/10.1161/CIRCRESAHA.110.223545

Gikas A, Sotiropoulos A, Pastromas V, Papazafiropoulou A, Apostolou O, Pappas S (2009) Seasonal variation in fasting glucose and HbA1c in patients with type 2 diabetes. *Prim. Care Diabetes* 111–114. https://doi.org/10.1016/j.pcd.2009.05.004

Gungor K, Gungor ND, Basar MM, Cengiz F, Ersahin SS, Cil K (2022) Relationship between serum vitamin D levels semen parameters and sperm DNA damage in men with unexplained infertility. *Eur. Rev. Med. Pharmacol. Sci.* **26**: 499–505. https://doi.org/10.26355/eurrev_202201_27875

Gunter T, Yule D, Gunter K, Elisee R, Salter J (2004) Calcium and mitochondria. *FEBS Lett.* **567**: 96–102. https://doi.org/10.1016/j.febslet.2004.03.071

Haq SH, AlAfaleq NO, Johari R Al (2019) Vitamin D treatment reverses the induced oxidative stress damage to DNA. *Pak. J. Biol. Sci.* **22**: 8–14. https://doi.org/10.3923/pjbs.2019.8.14

Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW (2014) SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* **14**: 43. https://doi.org/10.1186/1471-2288-14-43

Jensen M, Bjerrum P, Jessen T, Nielsen J, Joensen U, Olesen I, Petersen J, Juul A, Dissing S, Jorgensen N (2011) Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. *Hum. Reprod.* **26**: 1307–1317. https://doi.org/10.1093/humrep/der059

Jurutka P, Whitfield G, Hsieh J, Thompson P, Haussler C, Haussler M (2001) Molecular nature of the vitamin D receptorand its role in regulation of gene expression. *Rev. Endocr. Metab. Disord.* **2**: 203–216. https://doi.org/10.1023/a:1010062929140

Kállay E, Bareis P, Bajna E, Kriwanek S, Bonner E, Toyokuni S, Cross HS (2002) Vitamin D receptor activity and prevention of colonic hyperproliferation and oxidative stress. *Food Chem. Toxicol.* **40**: 1191–1196. https://doi.org/10.1016/s0278-6915(02)00030-3

Khrisnan A, Trump D, Johnson C (2012) The role of vitamin D in cancer prevention and treatment. *Rheum. Dis. Clin. North Am.* **38**: 161–78. https://doi.org/10.1016/j.rdc.2012.03.014

Krishnan A V., Feldman D (2010) Molecular pathways mediating the anti-inflammatory effects of calcitriol: Implications for prostate cancer chemoprevention and treatment. *Endocr. Relat. Cancer* **17**: 19–38. https://doi.org/10.1677/ERC-09-0139

Ladeira C, Pádua M, Veiga L, Viegas S, Carolino E, Gomes MC, Brito M (2015) Influence of serum levels of vitamins A, D, and E as well as vitamin D receptor polymorphisms on micronucleus frequencies and other biomarkers of genotoxicity in workers exposed to formaldehyde. *Lifestyle Genomics* **8**: 205–214. https://doi.org/10.1159/000444486

Lan N, Luo G, Yang X, Cheng Y, Zhang Y, Wang X, Wang X, Xie T, Li G, Liu Z (2014) 25-Hydroxyvitamin D3-deficiency enhances oxidative stress and corticosteroid resistance in severe asthma exacerbation. *PLoS One* **9**: e111599. https://doi.org/10.1371/journal.pone.0111599

Liana P, Liberty IA, Murti K, Hafy Z, Salim EM, Zulkarnain M, Umar TP (2022) A systematic review on neutrophil extracellular traps and its prognostication role in COVID-19 patients. *Immunol. Res.* **70**: 449–460. https://doi.org/10.1007/s12026-022-09293-w

Liu H, Feng X, Wu S, Zong T, Li B, Zhang, Zengli (2019) Vitamin D resists cyclophosphamide-induced genomic and DNA damage in CHL cells *in vitro* and in mice *in vivo*. *Nutr. Cancer* **71**: 1030–1039. https://doi.org/10.1080/01635581.2019.1595050

Machado C da S, Venancio VP, Aissa AF, Hernandes LC, de Mello MB, Del Lama JEC, Marzocchi-Machado CM, Bianchi MLP, Antunes LMG (2016) Vitamin D3 deficiency increases DNA damage and the oxidative burst of neutrophils in a hypertensive rat model. *Mutat. Res. Toxicol. Environ. Mutagen.* **798**: 19–26. https://doi.org/10.1016/j.mrgentox.2016.01.005

Machado CDS, Aissa AF, Ribeiro DL, Antune L, Greggi M (2019) Vitamin D supplementation alters the expression of genes associated with hypertension and did not induce DNA damage in rats. *J. Toxicol. Environ. Health* **82**: 299–313. https://doi.org/10.1080/15287394.2019.1592044

Maestro B, Molero S, Bajo S, Dávila N, Calle C (2002) Transcriptional activation of the human insulin receptor gene by 1,25-dihydroxyvi-

tamin D3. *Cell Biochem. Funct.* **20**: 227–232. https://doi.org/10.1002/cbf.951

Meerza D, Naseem I, Ahmed J (2012) Effect of 1, 25$(OH)_2$ vitamin D_3 on glucose homeostasis and DNA damage in type 2 diabetic mice. *J. Diabetes Complications* **26**: 363–368. https://doi.org/10.1016/j.jdiacomp.2012.05.013

Mehri N, Haddadi R, Ganji M, Shahidi S, Soleimani Asl S, Taheri Azandariani M, Ranjbar A (2020) Effects of vitamin D in an animal model of Alzheimer's disease: Behavioral assessment with biochemical investigation of hippocampus and serum. *Metab. Brain Dis.* **35**: 263–274. https://doi.org/10.1007/s11011-019-00529-7

Merino O, Sánchez R, Gregorio B, Sampaio F, Risopatrón J (2018) Effects of diet-induced obesity and deficient in vitamin D on spermatozoa function and DNA integrity in Sprague-Dawley rats. *Biomed Res Int.* **2018**: 5479057. https://doi.org/10.1155/2018/5479057

Migliaccio S, Di Nisio A, Magno S, Romano F, Barrea L, Colao AM, Muscogiuri G, Savastano S (2022) Vitamin D deficiency: a potential risk factor for cancer in obesity? *Int. J. Obes. (Lond).* **46**: 707–717. https://doi.org/10.1038/s41366-021-01045-4

Mohammed M, Aboulhoda B, Mahmoud R (2019) Vitamin D attenuates gentamicin-induced acute renal damage *via* prevention of oxidative stress and DNA damage. *Hum. Exp. Toxicol.* **38**: 321–335. https://doi.org/10.1177/0960327118812166

Myszka M, Klinger M (2014) The immunomodulatory role of Vitamin D. *Postepy Hig. Med. Dosw. (Online)* **68**: 865–878. https://doi.org/10.5604/17322693.1110168

Nair-Shalliker V, Armstrong BK, Fenech M (2012a) Does vitamin D protect against DNA damage? *Mutat. Res. – Fundam. Mol. Mech. Mutagen.* **733**: 50–57. https://doi.org/10.1016/j.mrfmmm.2012.02.005

Nair-Shalliker V, Fenech M, Forder PM, Clements MS, Armstrong BK (2012b) Sunlight and vitamin D affect DNA damage, cell division and cell death in human lymphocytes: a cross-sectional study in South Australia. *Mutagenesis* **27**: 609–614. https://doi.org/10.1093/mutage/ges026

Najeeb HA, Othman R, Salih SF, Mohammed AA, Ismaeel Q Al (2020) Vitamin D level and endogenous DNA damage in patients with cancers in Duhok city, KRG-Iraq. *Ann. Med. Surg.* **10**: 462–467. https://doi.org/10.1016/j.amsu.2020.10.065

Ng CY, Amini F, Ahmad Bustami N, Tan ESS, Tan PY, Mitra SR (2021) Association of DNA damage with vitamin D and hair heavy metals of obese women. *Mol. Cell. Toxicol.* **17**: 429–438. https://doi.org/10.1007/s13273-021-00149-2

O'Callaghan-Gordo C, Kogevinas M, Fthenou E, Pedersen M, Espinosa A, Chalkiadaki G, Daraki V, Dermitzaki E, Decordier I, Georgiou V, Merlo DF, Roumeliotaki T, Vande Loock K, Kleinjans J, Kirsch-Volders M, Chatzi L (2017) Vitamin D insufficient levels during pregnancy and micronuclei frequency in peripheral blood T lymphocytes mothers and newborns (*Rhea cohort*, Crete). *Clin. Nutr.* **36**: 1029–1035. https://doi.org/10.1016/j.clnu.2016.06.016

Osmancevic A, Sandström K, Gillstedt M, Landin-Wilhelmsen K, Larkö O, Wennberg Larkö A-M, F. Holick M, Krogstad A-L (2015) Vitamin D production after UVB exposure – A comparison of exposed skin regions. *J. Photochem. Photobiol. B Biol.* **143**: 38–43. https://doi.org/10.1016/j.jphotobiol.2014.12.026

Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A (2016) Rayyan – a web and mobile app for systematic reviews. *Syst. Rev.* **5**: 210. https://doi.org/10.1186/s13643-016-0384-4

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* **372**: n71. https://doi.org/10.1136/bmj.n71

Peng X, Vaishnav A, Murillo G, Alimirah F, Torres KEO, Mehta RG (2010) Protection against cellular stress by 25-hydroxyvitamin D3 in breast epithelial cells. *J. Cell. Biochem.* **110**: 1324–1333. https://doi.org/10.1002/jcb.22646

Petersen B, Wulf HC, Triguero-Mas M, Philipsen PA, Thieden E, Olsen P, Heydenreich J, Dadvand P, Basagaña X, Liljendahl TS, Harrison GI, Segerbäck D, Schmalwieser AW, Young AR, Nieuwenhuijsen MJ (2014) Sun and ski holidays improve vitamin D status, but are associated with high levels of DNA damage. *J. Invest. Dermatol.* **134**: 2806–2813. https://doi.org/10.1038/jid.2014.223

Potter TM, Neun BW, Stern. ST (2011) Assay to detect lipid peroxidation upon exposure to nanoparticles. *Mol. Biol.* **697**: 181–89

Qiao W, Yu S, Sun H, Chen L, Wang R, Wu X, Goltzman D, Miao D (2020) 1,25-Dihydroxyvitamin D insufficiency accelerates age-related bone loss by increasing oxidative stress and cell senescence. *Am. J. Transl. Res.* **12**: 507–518. PMID: 32194899

Siebert C, Santos T Dos, Bertó C, Parisi M, Coelho R, Manfredini V, Barbé-Tuana F, Wyse A (2018) Vitamin D supplementation reverses DNA damage and telomeres shortening caused by ovariectomy in hippocampus of wistar rats. *Neurotox. Res.* **34**: 538–546. https://doi.org/10.1007/s12640-018-9909-z

Smith SM, Zwart SR, Block G, Rice BL, Davis-Street JE (2005) The nutritional status of astronauts is altered after long-term space flight aboard the International Space Station. *J. Nutr.* **135**: 437–443. https://doi.org/10.1093/jn/135.3.437

Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, Henry D, Altman DG, Ansari MT, Boutron I, Carpenter JR, Chan AW, Churchill R, Deeks JJ, Hróbjartsson A, Kirkham J, Jüni P, Loke YK, Pigott TD, Ramsay CR, Regidor D, Rothstein HR, Sandhu L, Santaguida PL, Schünemann HJ, Shea B, Shrier I, Tugwell P, Turner L, Valentine JC, Waddington H, Waters E, Wells GA, Whiting PF, Higgins JP (2016) ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* **355**: i4919. https://doi.org/10.1136/bmj.i4919

Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng HY, Corbett MS, Eldridge SM, Emberson JR, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni P, Kirkham JJ, Lasserson T, Li T, McAleenan A, Reeves BC, Shepperd S, Shrier I, Stewart LA, Tilling K, White IR, Whiting PF, Higgins JPT (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* **366**: l4898. https://doi.org/10.1136/bmj.l4898

Talaei A, Mohamadi M, Adgi Z (2013) The effect of vitamin D on insulin resistance in patients with type 2 diabetes. *Diabetol. Metab. Syndr.* **5**: 8. https://doi.org/10.1186/1758-5996-5-8

Tilstra JS, Robinson AR, Wang J, Gregg SQ, Clauson CL, Reay DP, Nasto LA, St Croix CM, Usas A, Vo N, Huard J, Clemens PR, Stolz DB, Guttridge DC, Watkins SC, Garinis GA, Wang Y, Niedernhofer LJ, Robbins PD (2012) NF-κB inhibition delays DNA damage-induced senescence and aging in mice. *J. Clin. Invest.* **122**: 2601–2612. https://doi.org/10.1172/JCI45785

Umar TP, Samudra MG, Nashor KMN, Agustini D, Syakurah RA (2022) Health professional student's volunteering activities during the COVID-19 pandemic: A systematic literature review. *Front. Med.* **9**: 797153. https://doi.org/10.3389/fmed.2022.797153

Umar TP, Siburian R (2022) Routine laboratory testing role for Covid-19 identification: A systematic review. *Med. Sci. J. Adv. Res.* **3**: 99–106. https://doi.org/10.46966/msjar.v3i3.49

Usman M, Woloshynowych M, Britto JC, Bilkevic I, Glassar B, Chapman S, Ford-Adams ME, Desai A, Bain M, Tewfik I, Volpi E V. (2021) Obesity, oxidative DNA damage and vitamin D as predictors of genomic instability in children and adolescents. *Int. J. Obes.* **45**: 2095–2107. https://doi.org/10.1038/s41366-021-00879-2

Vuolo L, Di Somma C, Faggiano A, Colao A (2012) Vitamin D and cancer. *Front. Endocrinol. (Lausanne)* **3**: 1–13. https://doi.org/10.3389/fendo.2012.00058

Wacker M, Holiack MF (2013) Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients* **5**: 111–148. https://doi.org/10.3390/nu5010111

Wang EW, Collins AR, Pang MYC, Siu PPM, Lai CKY, Woo J, Benzie IFF (2016) Vitamin D and oxidation-induced DNA damage: is there a connection? *Mutagenesis* **31**: 655–659. https://doi.org/10.1093/mutage/gew033

Wenclewska S, Szymczak-Pajor I, Drzewoski J, Bunk M, A Śliwińska (2019) Vitamin D supplementation reduces both oxidative DNA damage and insulin resistance in the Elderly with metabolic disorders. *Int J Mol Sci* **20**: 2891. https://doi.org/10.3390/ijms20122891

Yavuzer H, Yavuzer S, Cengiz M, Erman H, Doventas A, Balci H, Erdincler DS, Uzun H (2016) Biomarkers of lipid peroxidation related to hypertension in aging. *Hypertens. Res.* **39**: 342–348. https://doi.org/10.1038/hr.2015.156